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10/28/03  
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Hi, I need the following references please:

Farber et al. PLANT PIGMENTS AS COLORANTS IN CANE SUGAR, proceeding 1972 Tech. Sess. Cane Sugar Refining Research p.23.

Marry Ann Godshall et al (please note, this may be 'Goodshall') PHENOLICS IN SUGAR PRODUCTS, proceeding of the 1982 sugar processing conference, p.47.

Margaret A. Clark et al., STRUCTURE OF COLORANTS, proceeding of the 1988 sugar processing research conference, 1988, p.183.

Richard Riffer, NON-SUGAR AND SUGAR REFINING-HANDBOOK OF SUGAR REFINING; (2000) John Wiley & sons, Inc, NY.

Judy McBride CAN FOODS FORESTALL AGING, 2/1999, Agric. Research Magazine, USDA.

TI New flavonoids from sugarcane (Saccharum);  
isolation and structure determination of tricetin 7-(2"-rhamnosyl)-  
alpha-galacturonide and orientin 7,3'-dimethyl ether  
AU Mabry T J; Liu Y L; Declamorica G; Chopin J; Markham K R; Paton N H  
CS CSR  
LO The Department of Botany, The University of Texas at Austin, TX 78712,  
USA.  
SO J.Nat.Prod.; (1984) 47, 1, 127-30  
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DT Journal  
LA English

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(P)

# STRUCTURE OF COLORANTS

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(84)

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## INTRODUCTION

(92) US

The classification of colorant compounds in both cane and beet sugar manufacture into plant-derived and process-generated or factory-generated groups is well known. Compounds that originate in the growing plant include flavonoids, substituted phenolics, polyphenolics, amines and amino acids; colorant molecules are found in these groups or in their reaction products. Compounds of plant origin have been further classified by molecular weight range and by their tendency to be occluded preferentially in the sugar crystal (Clarke et al., 1984; Clarke et al., 1987; Kofod-Neilsen et al., 1980; Mantovani et al., 1985; Shore et al., 1984; Smith and Gregory, 1971; Tu et al., 1977).

Recently, work at S.P.R.I. has concentrated on a group of cane sugar colorants of very high molecular weight, of plant origin that are intractable in process--most factory and refinery processes fail to remove a significant amount of this group, which continues through process to end up in the sugar crystal. This type of compound shows both polysaccharide characteristics and visible color (Clarke et al., 1987; Godshall et al., 1987).

Although this type of color is not intense, it is expensive to the processor: It has reduced the efficiency of every process in the factory and refinery without being eliminated, while consuming energy and process chemicals, and so has lowered the overall efficiency of production.

In this paper, the structure of this type of polysaccharide-colorant will be discussed. Similar compounds have been found in plants closely related to sugarcane, and potential polysaccharide-colorant complex molecules have been reported in sugarbeet.

## PLANT CELL WALL POLYSACCHARIDES

The polysaccharide known as I.S.P. (Indigenous Sugarcane Polysaccharide) is an arabinogalactan, with glucuronic acid residues (Roberts et al., 1976). I.S.P. is soluble and travels through cane sugar processing to end up in even the highest quality refined white sugar (Roberts and Godshall, 1978).

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There has been considerable research into the cell wall polysaccharides in corn and wheat, both members of the Gramineae family to which sugarcane belongs. In corn and wheat, the pentosans are also important as polysaccharides in end product. In sugarcane, the major pentosan is a polymer of xylan, the main component of bagasse, the fibrous residue of cane. Because traces of xylose are often found with I.S.P., it is not clear whether soluble pentosans exist in sugarcane, or whether there is some xylose in the glucuronoarabinogalactan (I.S.P.) structure. The latter is the more likely case.

The major cell wall polysaccharides in the grasses are this type of compound and glucans. The polysaccharides are cross-linked by a variety of mechanisms to provide stability for the cell-wall structure. These macromolecular associations probably include glycosidic and non-glycosidic covalent linkages, hydrogen bonds, ionic connection and a variety of intramolecular attractive forces; structure elucidation is in the early stages. However, it is known that some small substituted phenolics can serve as cross-linking components between cell wall chains. One of these is ferulic acid, shown in Figure 1. This, in corn, is esterified to the O-2 position of the arabinose residues (Nevins and Kato, 1984), as shown in Figure 2. Ferulic acid can cross-link in the plant by dimerization, catalyzed by peroxidase in the presence of  $H_2O$ , as shown in Figure 3.

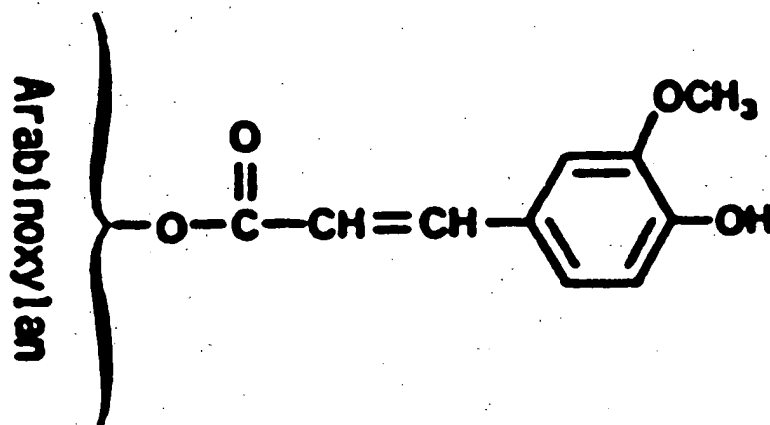


Figure 1.--Ferulic acid esterified onto arabinoxylan backbone in corn.

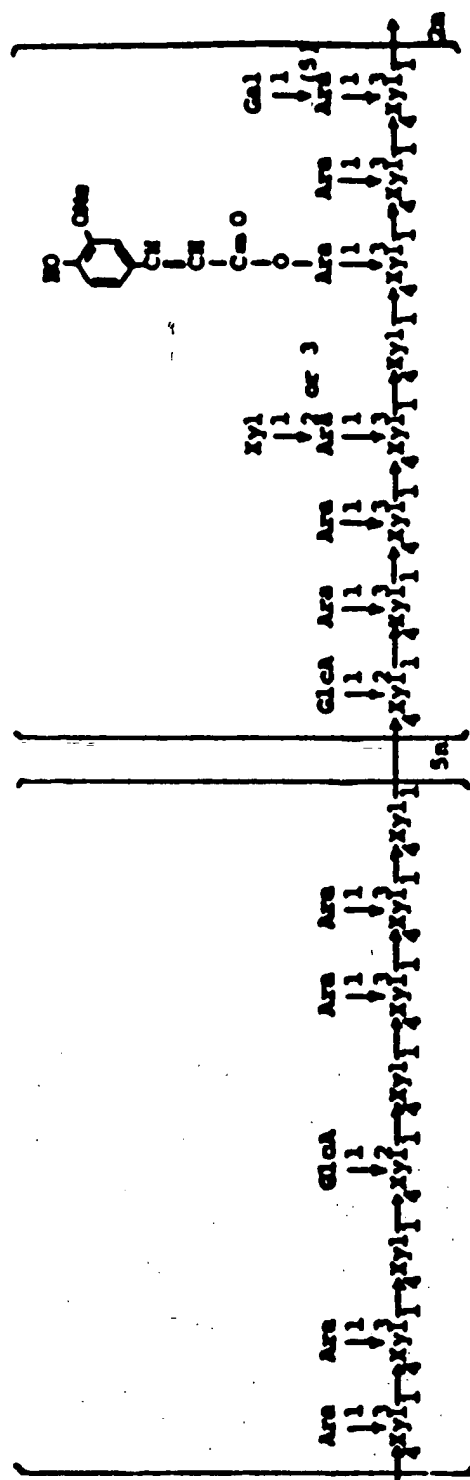


Figure 2.--Corn glucuronoxarabino xylan backbone with ferulic acid ester.

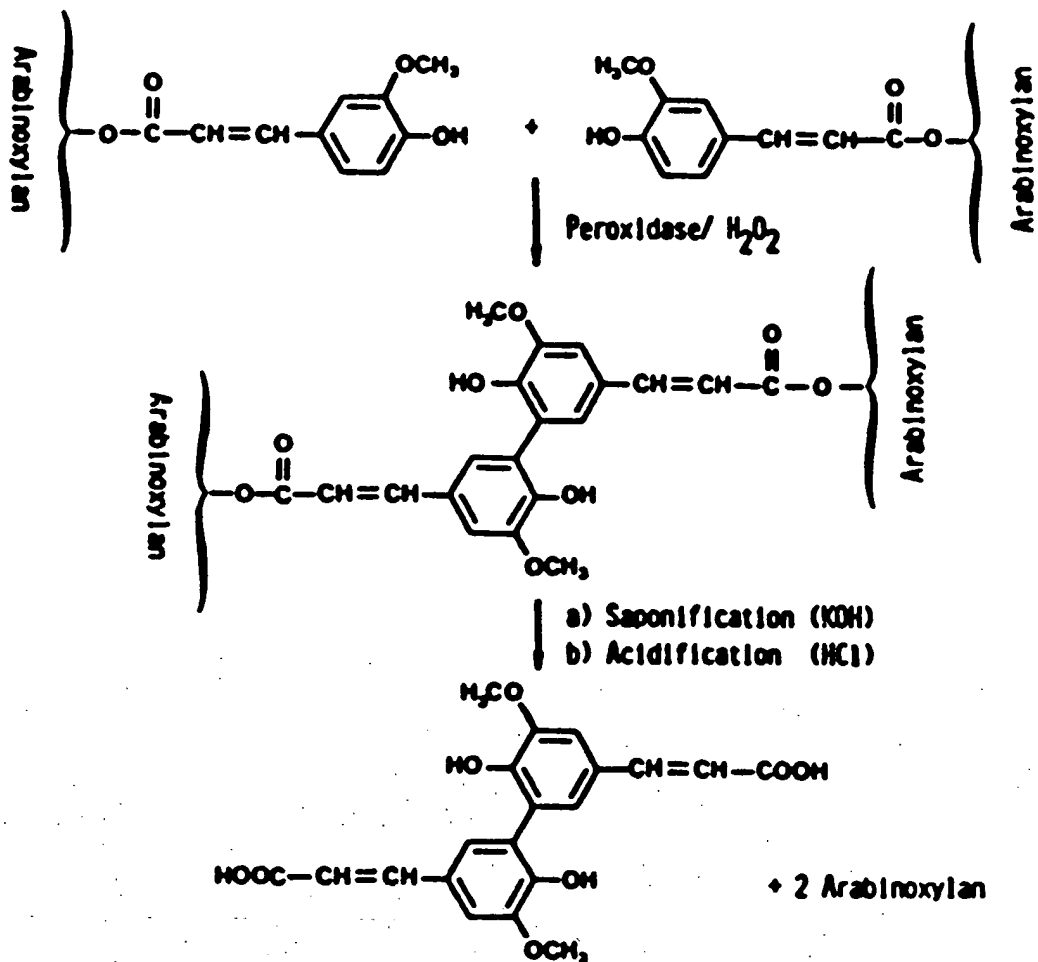


Figure 3.--Dimerization of ferulic acid by peroxidase enzymes to create cross-linking.

These cross-linkages are easily broken when the plant undergoes processing and part of the cell wall polysaccharides are solubilized. However, the ferulic acid ester linkage is less easily hydrolyzed and remains attached to the backbone even when the polysaccharide is degraded into small units. It is proposed (Nevins and Kato, 1985) that the phenolic acid preserves the glycosidic linkages in the immediate vicinity of its point of attachment and prevents enzymic hydrolysis.

To relate this to sugarcane and cane sugar processing: Ferulic acid is a phenolic constituent that is always observed in raw and refined sugars (Godshall and Roberts, 1982). The constituents in these studies are identified after acid or base extraction which could break the ester linkage and release ferulic acid. Ferulic acid is a very pale yellow active compound that can readily react in sugar to form darker colored compounds. In studies on behavior of colorant components model systems for sugar boiling, Devereux (1980) found that when ferulic acid was added to a white sugar liquor, crystals from that liquor developed a yellow color after only a few days, thereby identifying ferulic acid as a compound that causes sugar to darken in storage.

Roberts also noted (Roberts and Godshall, 1978), in a study on the behavior of high molecular weight components in sugar refining, that material that appeared to be polysaccharide, isolated from refined sugar, when treated with ethanol, released some colored compounds. The observation indicates that some colorant can travel through all raw sugar factory and refinery processes while associated with polysaccharide covalently attached, probably since hydrogen bonding or other non-bonded interactions seem unlikely to resist the prevalent pH and temperature conditions. These observations can be explained by the presence of phenolic acid groups esterified to cell wall polysaccharides.

#### BEET SUGAR COLORANT

For many years, polysaccharides in sugarbeet and beet sugar have been classified as pectins, and relatively little work has been done on them until recently. The advent of sugarbeet fiber as an important dietary fiber has spurred further research. J. F. Thibault, at INRA, at Nantes, in France, has made a systematic study of beet pectin (Thibault, 1988).

Beet pectin is a soluble polysaccharide, composed primarily of galacturonic acid and monosaccharide residues. Rombouts and Thibault (1983) have recently identified ferulic acid groups, esterified onto galacturonic acid units of the rhamnogalacturonan backbone. These are analogous to the abovementioned ferulic acid-hemicellulose complex; ferulic acid in sugarcane could be esterified onto the glucuronic acid residues of ISP.

It is proposed, therefore, that some colorant and color precursors in both cane and beet factories travel through process attached to polysaccharide; the complex is soluble; in crystallization it preferentially enters the crystal and could, upon storage, especially in conditions of high temperature, de-esterify to release colorant. The de-esterification might also occur in process; high alkalinity is likely to break the ester linkage. The colorant thus formed is no longer associated with polysaccharide and so less likely to cocrystallize with sucrose. It has often been observed that, in a comparison of fine liquors from carbonatation and phosphatation processes, sugars of equal whiteness can be crystallized from carbonatated liquor of much higher color than phosphatated. Carbonatation conditions use a pH high enough to hydrolyze the ferulic acid groups off the polysaccharide, whereas phosphatation conditions do not. Hydrolyzed phenolic acids would contribute to syrup or liquor color, but less to crystal color. It is therefore possible that this type of colorant provides one contributing factor to the observed differences in crystallization behavior from carbonatation and phosphatation liquors. Alkaline decomposition of invert also contributes to this phenomenon, of course, and a quantitative study is required to determine relative contributions.

Preliminary work at S.P.R.I. on high molecular weight color and polysaccharide behavior in beet juice with varying carbonatation conditions shows that at higher pH (11), more total color is removed than at lower pH (10), but a higher proportion of remaining color is low molecular weight at pH 11 than at pH 10. Some of this color, as abovementioned, comes from invert decomposition, but some may be de-esterified phenolic colorant.

It is also possible that cross-linked hemicellulose, as shown in Figure 3, is a part of the polysaccharide-colorant complex material.

Other workers have recently emphasized the growing importance of high molecular weight colorant in beet sugar. Broughton et al in 1987 reported observations on HPLC separation systems of colorant at 40,000 daltons. It was suggested that this colorant is formed in process from polymerization of smaller molecules.

Vogel and Schiweck (1988), of Süddeutsche Zucker, have recently begun an investigation into high molecular weight material in sugarbeet, and reported from 0.13% - 0.14% by weight of polysaccharide material greater than 10,000 daltons. In their gel permeation studies, a peak at 100,000 daltons was observed in thin and thick juice and molasses, and another peak at 200,000 daltons in raw juice, both with colorant and polysaccharide nature. These were attributed to hemicellulose from the beet.

## CONCLUSION

It appears that high molecular weight material from both sugarcane and sugarbeet is of importance in processing both for its polysaccharide characteristics and its colorant properties. Recent observations indicate that this material, known for some years to be a factor in sugarcane processing, may also affect sugarbeet processing.

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## DISCUSSION

Question: A comment, rather than a question. I agree with Dr. Clarke that there are a number of colorants of interest to both beet and cane. In the 1987 campaign, we carried out in Ferrara, in cooperation with the sugar company Eridania, some investigations concerning color precursors. To our surprise, we found, along with polyphenolics in sugarbeet juices, also flavonoids--apigenin and other compounds. This work was published by Dr. Maurandi (Indus. Sucr. Ital. 1988 (3)).

Clarke: That paper contains some very good work.

Question: You talked about the combination of colorant and polysaccharide at high molecular weight which must be in the millions. How do you believe that such an enormous molecule can be preferentially taken into the sucrose crystal?

Clarke: I can certainly explain how any complex that has a dextran in it can be taken into the crystal. The sucrose molecule, that is the beginning of any dextran molecule, will fit right into the sucrose lattice. Other glucans have at least glucose as one terminal group.

Question: A couple of points: First, on the color of feruloylate esters--it's easy to believe that water-soluble polymers carrying feruloylate esters can be extracted from Gramineae. On the question of their color--this will be extremely sensitive to pH.

When the parahydroxy group ionizes, you get an extended sequence of conjugated double bonds, and therefore color. That's the yellow color that you always get with paraphenols of the cinnamic acid group. So when you get materials showing more color in alkali, you suspect the parahydroxy group.

In answer to another question, the proanthocyanidins are colorless, the same as leucoanthocyanins, but develop red to orange colors in acid.

Clarke: Those compounds have been found in raw sugar. It seems to me we've found these involved in plant protection systems also--perhaps Mrs. Godshall will comment on this.

Godshall, S.P.R.I.: We have found luteolinidin (3-deoxyanthocyanin)--that is orange-colored. We also did some work that was never published, some years ago, on leucoanthocyanins in cane juice. We were able to extract them and, upon acidification, obtain a red color. Perhaps we should follow up on this--the compounds are definitely in cane juice.

Clarke: This all emphasizes that both cane juice and beet juice contain plant extracts in very complex systems.